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Pattern Formation in *Hydra vulgaris* Is Controlled by Lithium-Sensitive Processes

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Application of lithium ions leads to dramatic alterations in the positional value and subsequently to the formation of ectopic foot or head structures in *Hydra vulgaris*. Prolonged LiCl treatment decreases and pulse treatment increases the positional value. The decrease in the positional value is manifested in the formation of multiple ectopic feet along the body axis of intact animals or regenerates. Regeneration experiments reveal that long term application of the ion leads to transformation of prospective head into foot tissue. This transformation requires lithium pretreatment and exposure of the excised pieces to lithium for a further 5–8 hr. Pulse treatment of hydra with lithium elevates the positional value. Regenerating distal thirds differentiate ectopic head structures instead of a foot. Changes in the lithium concentration during the experiment lead to the differentiation of ectopic heads in the upper as well as ectopic feet in the lower body region. The characteristics of pattern respecification presented in this paper suggest that lithium interferes with the main pattern forming system(s) in hydra.

INTRODUCTION

Pattern formation and regeneration of missing structures are rigidly controlled in the freshwater polyp *Hydra*. According to experimental data, head and foot formation are dependent on two pairs of developmental gradients each consisting of an activating and an inhibitory component (reviewed in Meinhardt, 1982; Bode and Bode, 1984; Javois, 1992). The activating component determines which structure is formed, whereas the inhibitory component suppresses the formation of additional structures in close proximity. Head activating and head inhibiting potentials both peak in the head region (high positional value) and decrease toward the foot region (low positional value) (Wolpert *et al.*, 1974). Foot activating and foot inhibiting potentials run in the opposite direction.

Several agents have been shown to perturb pattern formation in hydra and cause the formation of fully functional ectopic structures in intact animals. Ectopic heads or feet are induced by treatment with lithium ions (Yasugi, 1974; Hassel and Berking, 1989, 1990), oligomycin (Hornbruch and Wolpert, 1975), or diacylglycerol (Müller, 1989). Diacylglycerol (DG) apparently elevates the positional value in a wave-like fashion along the body axis and thus leads to multiple ectopic heads. Lithium ions either decrease the positional value and lead to the formation of (multiple) ectopic feet or, dependent on the treatment schedule, possibly increase the positional value (Hassel and Berking, 1990). Lithium ions have been known as a vegetalizing agent for a long time (Herbst, 1892) and have recently been shown to affect cell fate determination in the sea urchin (Nocente-McGrath *et al.*, 1991), in frog embryos (Kao *et al.*, 1986), and in the slime mold *Dictyostelium discoideum* (Van Lookeren Campagne *et al.*, 1988). Most intriguing is the effect on embryonic development in *Xenopus*, where lithium leads to the dorsalization of embryos (Kao and Elinson, 1989). *Xenopus* is protected against the teratogenic effect of lithium if inositol is applied simultaneously (Busa and Gimlich, 1989). This has led to the hypothesis that lithium affects embryogenesis by disrupting inductive interactions mediated by second messenger pathways (reviewed in Berridge *et al.*, 1989). It appears likely that pattern formation in hydra involves second messenger systems as two of the morphogenetically active agents affect second messenger metabolism. Diacylglycerol is a second messenger of the phosphatidylinositol cycle (Berridge, 1986), and lithium ions are known to interfere with second messenger metabolism in a variety of cells (Berridge *et al.*, 1989).

We previously described the dramatic effects of lithium ions on morphology and cell composition in hydra (Hassel and Berking, 1989, 1990). The aim of the present study was to investigate if lithium ions are able to respecify the pattern of hydra during regeneration and if this respecification follows the rules described for pattern formation in the animal.

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MATERIALS AND METHODS

Culture conditions. *Hydra vulgaris* (P. Tardent, Zürich) were kept in hydra medium as mass cultures according to Bode *et al.* (1973) and fed five times per week with freshly hatched nauplii of *Artemia salina.* The experiments were carried out in petri dishes at a density of one to two hydra per milliliter. Our medium consisted of 0.01 m*M* Tris, 1 m*M* $CaCl_2$, 1 m*M* NaCl, 0.1 m*M* KCl, 0.01 m*M* $MgCl_2$, and 0.02 m*M* EDTA-Na_2 dissolved in spring water. The experiments always started after the first feeding following 2 days of starvation. A constant feeding scheme is critical for the lithium effects: unfed animals are much more susceptible to the influence of the ion and the results are quantitatively not comparable if one uses animals grown under different culture conditions. LiCl was dissolved in hydra medium, and no contact with normal medium was allowed during the long term treatment.

Regeneration and identification of regenerated structures. For regeneration, budless animals were starved for 24 hr before cutting. Cut fragments were kept separately in petri dishes, and medium was changed daily. During regeneration the animals were not fed. Fragments were classified by the relative position of cutting in relaxed animals. Zero percent body length corresponds to the basal disc, 100% body length to the hypostome. For most of the experiments hydra were cut into thirds, resulting in lower or proximal (0–33%), gastric (33–66%), and upper or distal (66–100%) fragments.

Ectopic feet were identified by morphological criteria and using the foot-specific peroxidase staining developed by Hoffmeister and Schaller (1985). Head structures were identified by morphological and functional criteria: developing tentacles had to contain battery cells and nematocytes and had to be able to capture and kill prey. All experiments were repeated twice unless otherwise indicated.

Statistical evaluation. Statistical analysis was carried out using Student's *t* test, leading to *p* values. The error bars represent the standard error of the mean.

RESULTS

Prolonged Lithium Treatment Accelerates Foot Regeneration and Promotes the Formation of Ectopic Feet

As previous experiments had shown that lithium ions applied as a long term treatment induced ectopic feet in intact animals (Hassel und Berking, 1990), we tested if head or foot regeneration were affected by the ion. Animals were pretreated in lithium for 1–4 days and cut into thirds. Regeneration proceeded in lithium for a further 1–4 days.

Lithium ions applied before and during regeneration inhibit head formation and stimulate foot formation in gastric and proximal fragments (Figs. 1a, 1c). After 1 day of pretreatment, head regeneration was inhibited in 70% of the regenerates, after 2 days in 90%, and after 3 days in 100% of the fragments. Concomitantly, an increasing number of animals differentiated foot tissue from the prospective head region (Figs. 1b, 1d; Fig. 2), thus forming bipolar foot regenerates (termed "bipolar feet"). Foot regeneration was slightly accelerated ($p < 0.05$) in gastric fragments of Li^+-treated animals (data not shown).

The yield of bipolar feet increased with the duration of pretreatment, indicating a decreasing positional value. After 1 day of pretreatment, 34% of the gastric thirds differentiated foot tissue instead of a head, the percentage increased to more than 70% after 2 days and almost 100% after more than 3 days of pretreatment. Thirds that contained the original foot completely failed to differentiate heads after only 2 days of treatment. Nevertheless, even 3 days of pretreatment led to only 30% bipolar feet; the remaining animals failed to develop any structure.

The bipolar feet were stable and persisted for at least 4 weeks. Budding restarted in some cases between the two feet, and the outgrowing bud then developed as a secondary axis and took over the functions of the missing head. The upper fragment regenerated a foot without abnormalities.

To monitor if a decrease in positional value also occurred in the upper half of the animals, where the head activating potential is highest, hydra were pretreated as in the first set of experiments and cut at 50, 70, and 90% body length. The regeneration of the 50–70 and 70–90 fragments was evaluated after 4 days of regeneration. Both regions developed up to 100% bipolar feet after 4 days of pretreatment (Fig. 3). The ability to regenerate a head decreased more slowly in the 70–90 than in the 50–70 fragments. After 2 days of lithium pretreatment, almost 30% of the 70–90 fragments still regenerated a head, but none of the 50–70 fragments. Even after 3 days in lithium, 10% of the 70–90 fragments were able to regenerate a head.

The experiment shows that the head forming potential persists longer in apical fragments, whereas bipolar foot formation occurs earlier in fragments more distant from the head.

The Yield of Ectopic Feet Depends on the Presence of a Foot, the Size of Regenerates, and Their Axial Position

Table 1 shows the results of a series of experiments in which fragment size and position were varied. The results indicate that these parameters as well as the pres-

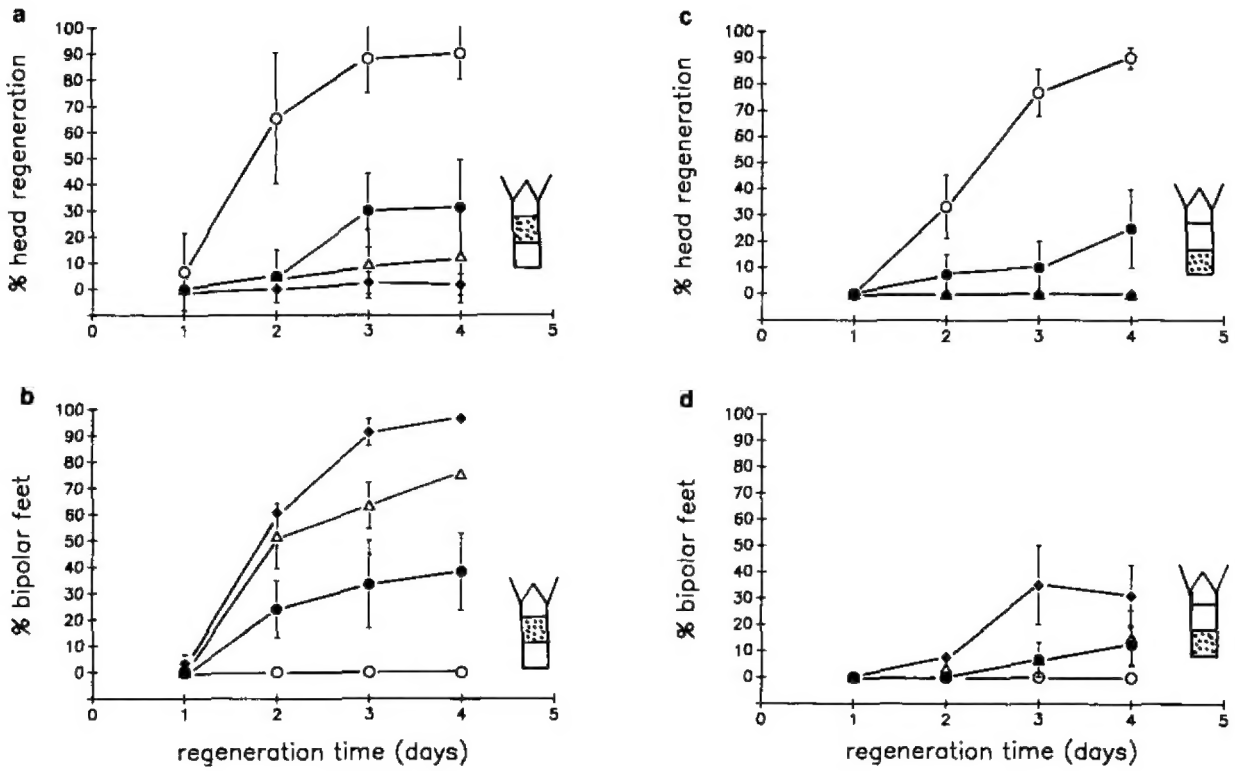

FIG. 1. Time course of head regeneration and formation of bipolar feet as a result of lithium pretreatment. Forty animals per time point were pretreated in 1 m*M* LiCl (untreated control ○, 1 day ●, 2 days △, 3–4 days ♦) and cut in thirds. The experiment was repeated twice. Regeneration proceeded in LiCl for further 1–4 days. (a) Head regeneration at the middle fragment, (b) formation of bipolar feet at the middle fragment, (c) head regeneration at the lower third, (d) formation of bipolar feet at the lower third.

ence of a foot influence the extent of head regeneration and the yield of ectopic feet. As fragments containing the original foot failed to differentiate large numbers of bipolar feet (Fig. 1d), we tested whether foot inhibition persisted despite the high foot activation potential (Table 1). Proximal thirds (0–30 fragments) formed 28% bipolar feet; removal of the foot (10–30 fragments) increased the yield of bipolar feet to 89%. The same effect

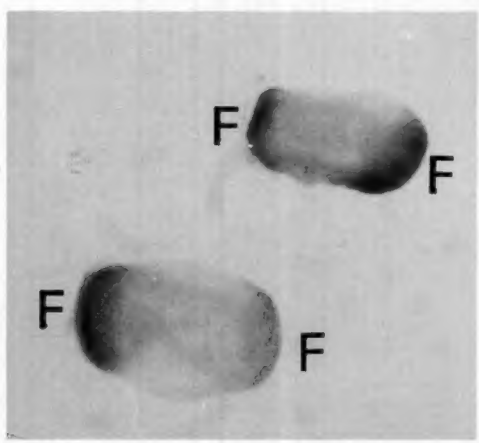

FIG. 2. Bipolar feet formed from the gastric third during regeneration in LiCl. These animal was pretreated for 4 days in 1 m*M* LiCl and cut. Regeneration proceeded in LiCl for a further 4 days. The developing feet were stained with the foot-specific peroxidase staining.

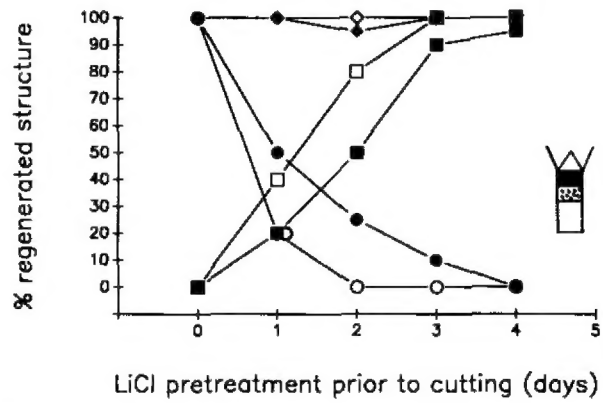

FIG. 3. Head and foot regeneration in the apical half during lithium treatment. Thirty animals per time point were treated with 1 m*M* LiCl as indicated and cut at 50, 70, and 90% body length. Regeneration proceeded in lithium for a further 4 days. 50–70% fragment: open symbols, 70–90% fragment: closed symbols. Head regeneration (○, ●), foot regeneration (△, ▲), bipolar foot formation (□, ■).

TABLE 1

INFLUENCE OF FRAGMENT SIZE ON THE YIELD OF BIPOLAR FEET IN DIFFERENT BODY REGIONS WITH OR WITHOUT REMOVAL OF HEAD AND/OR FOOT (N = 108–132) FOLLOWING LITHIUM TREATMENT

Fragment (% body length)	% differentiated structure				
	Additional lateral feet	Bipolar feet	"Head attempt"	Normal head	No structure
0–30	0	28	0	0	72
10–30	3	89	0	0	11
0–90	22	0	94[a]	6	0
10–90	86	5	90[a]	5	0
30–90	41	18	82	0	0
30–60	42	88	0	0	12
60–90	46	18	56	0	26

Note. Animals were treated in 1 m*M* LiCl for 3 days and cut as indicated. Regeneration proceeded for a further 4 days in 1 m*M* LiCl.

[a] Rudimentary heads are summarized under "head attempt" (see text).

could be observed with the long 0–90 fragments, which formed no bipolar feet, but 22% lateral patch feet; removal of the foot (10–90 fragments) increased the yield of lateral patch feet to 86%, and even a few bipolar feet differentiated. Thus foot inhibition apparently persists under the influence of lithium, and full foot activation (scored as bipolar and patch feet) is only attained if the source of foot inhibition is removed.

Small tissue pieces differentiated preferentially ectopic feet, whereas long segments regenerated rudimentary heads. Lithium-treated animals cut at 90% body length (just below the tentacles) did not form bipolar feet, although one-fifth of the animals developed lateral patch feet. Nevertheless, a normal head differentiated in only 6% of the animals, and 60% instead formed one or two protrusions ("head attempts") above the uppermost lateral foot (Fig. 4). This protrusion resembled in most cases a long cone with a very short central tentacle. An additional 35% of the regenerates formed rudimentary heads, much too small for a normal head. Fragments without a foot (cut at 10 and 90% body length) formed almost exclusively rudimentary heads with three to five tentacles. Differentiation of tentacle-specific cell types (*battery cells and nematocytes*) was observed in a few cases.

By decreasing the size of the fragments, the yield of bipolar feet increased. Setting the cut at 30 and 90% body length left relatively long fragments without head and foot. About 20% of these animals developed bipolar feet, the remaining 80% formed the above described "head attempts." The yield of lateral feet reached 40% with a maximum of seven feet per animal (Fig. 4). In comparison, almost 90% of the shorter 30–60 fragments differentiated bipolar feet, and 40–50% developed additional lateral patch feet. None of the 30–60 fragments made a "head attempt." Equivalent fragments from more distal positions (60–90% body length) formed only 18% bipolar feet.

In summary, this set of experiments shows that after 3 days of lithium treatment there is a weak, residual head formation potential in long fragments. By comparison, short fragments of proximal tissue form primarily bipolar feet and lateral feet. This tendency is enhanced in fragments without a foot.

Lithium Pre- Plus Post-treatment Is Necessary for the Differentiation of Bipolar Feet during Regeneration

To test whether post-treatment alone was sufficient for ectopic foot formation, hydra were cut into thirds without pretreatment and transferred within 10 min to 1 m*M* LiCl. Incubation in lithium was then carried out for 2 to 96 hr. Evaluation of this experiment after 4 days showed that no bipolar feet differentiated, although the head regeneration potential decreased rapidly (Fig. 5). After 8 hr of lithium treatment only 80% of the gastric thirds and 60% of the lower thirds regenerated a head. The head regeneration potential decreased further within the next 3 days, although unknown factors led to deviations of up to 20% between different time points.

The capacity for foot regeneration remained almost stable. Nevertheless 100% foot differentiation was not obtained. Up to 19% of the upper thirds (and only these)

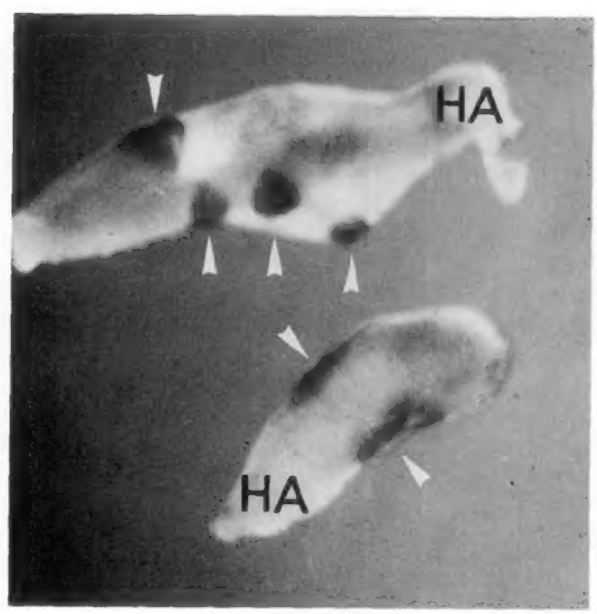

FIG. 4. Animals with multiple feet (arrows) and "head attempt" (HA) formed after pretreatment in lithium for 3 days and regeneration of fragments cut at 30 and 90% body length.

instead formed ectopic tentacles, and about 10% of the gastric thirds failed to form any structure. The decline in the capacity to regenerate a head or a foot after 96 hr of LiCl treatment probably does not reflect a general inhibition of differentiation as 16% of the upper thirds still differentiated ectopic tentacles. These ectopic tentacles were reduced within 4–8 days and finally replaced by a normal foot.

To test whether pretreatment alone was sufficient to induce the formation of bipolar feet or whether both pre- and post-treatment were necessary, animals were treated in 1 m*M* LiCl for 3 days, cut in thirds, and transferred directly to hydra medium or incubated further in LiCl. Regeneration was evaluated 3 days after cutting. Animals that had been transferred to normal medium within 5 hr after cutting regenerated almost normally: no ectopic feet formed, although head regeneration was inhibited to some extent in different experiments (Figs. 6a, 6b). If lithium treatment was continued for more than 6 hr after cutting, an increasing percentage of the animals differentiated bipolar feet.

After 6 hr of post-treatment, only one-third of the lower and gastric fragments were able to regenerate a head and some animals (1–5%) differentiated bipolar feet. Mid gastric fragments incubated for more than 24 hr after cutting formed almost exclusively bipolar feet.

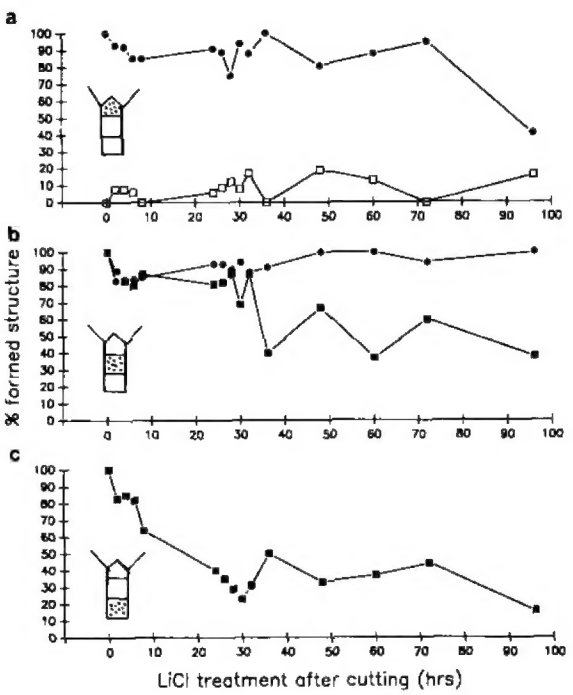

FIG. 5. Influence of lithium ions applied exclusively after sectioning. Animals (n = 80–130 per time point) were cut into thirds in three independent experiments and incubated in 1 m*M* LiCl for the times indicated. Evaluation of all experiments was carried out at 96 hr. Foot regeneration (●), head regeneration (■), ectopic tentacles (□).

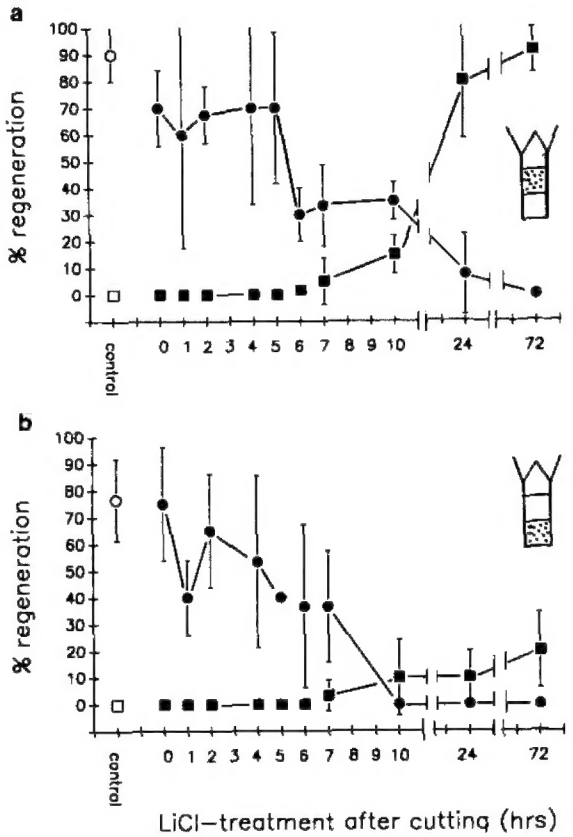

FIG. 6. Regeneration of the middle and lower third after various times of LiCl post-treatment. Thirty to forty animals per time point were pretreated for 3 days in 1 m*M* LiCl, cut into thirds, and treated further in LiCl as indicated. Regeneration was evaluated after 3 days. (a) gastric third, (b) lower third: head regeneration (○, ●), formation of bipolar feet (□, ■).

Head formation capacity decreased much faster in the lower body region than in the upper region. Head regeneration in the lower third was completely suppressed after 10 hr of post-treatment compared to 72 hr in the middle third. The lower thirds differentiated fewer bipolar feet than gastric fragments. These results are in accordance with the results presented in Fig. 1 and Table 1 and probably reflect the residual head activation and foot inhibition potentials.

Post-treatment with 1 m*M* lithium had an additional remarkable effect: 7% of the upper thirds (n = 388) developed a central tentacle instead of a foot, or additional lateral tentacles close to the presumptive foot end (Fig. 7). These ectopic tentacles contained battery cells filled with all types of nematocytes like normal tentacles and were only transiently stable. In control animals, the formation of ectopic tentacles did not occur.

Taken together, the experiments show that pre- plus post-treatment is necessary to induce ectopic feet. Pretreatment alone has very little effect. Post-treatment

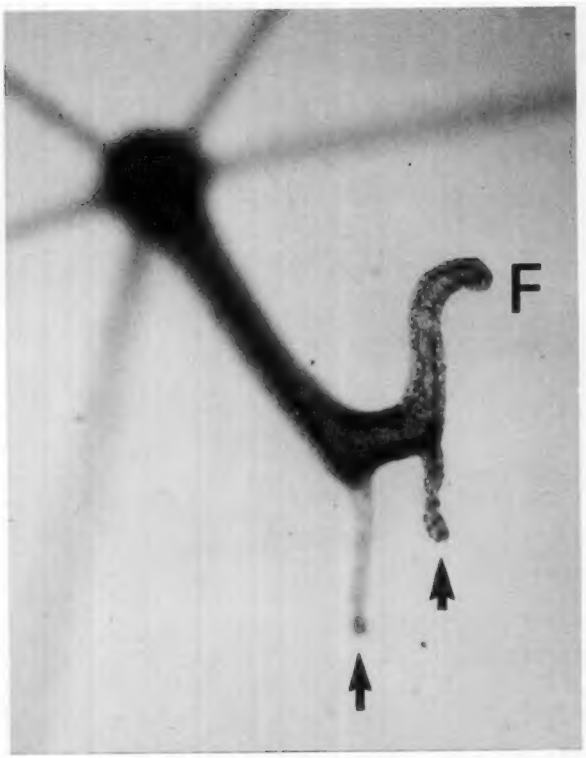

FIG. 7. Ectopic tentacles formed in a regenerating head fragment. This animal was pretreated for 3 days in 1 m*M* LiCl and allowed to regenerate for a further 3 days in normal medium. Two ectopic tentacles (arrows) developed in addition to a normal foot (F).

alone can inhibit head regeneration, although bipolar feet differentiate only if the ion is present for more than 6 hr.

Pulse Treatment with LiCl Induces the Formation of Ectopic Head Structures

Previous experiments (Hassel and Berking, 1990) have shown that pulse treatment with high lithium concentrations inhibited the detachment of buds from the parent animals. This phenomenon was interpreted as an elevation of the positional value which manifested itself in the failure to form a foot at the proper position. To test whether the positional value is actually increased by a lithium pulse, hydra (n = 240) were treated for 4 hr with 10 m*M* LiCl, cut into thirds, and allowed to regenerate in normal medium. There was no significant difference in the time needed to regenerate a head or a foot (data not shown), but up to 35% of the upper and middle thirds completely failed to regenerate a foot (Table 2). In addition, 19% of these animals formed ectopic tentacles, which differentiated, as in the long term experiments (Fig. 7), exclusively at the upper third opposite or lateral to the existing head. The tentacles showed normal morphology and did not prohibit the formation of feet in neighboring tissue.

Reducing the LiCl Concentration during Long Term Treatment Increases Both Head and Foot Forming Potentials

In order to test if changes in the LiCl concentration during long term treatment could also shift the differentiation potential of intact and regenerating animals, several experiments were carried out in which the lithium concentration was varied between 1 and 4 m*M*. Figure 8 summarizes the treatment protocols and results. In about half of the animals, ectopic head structures differentiated in the upper body region, ectopic feet in the lower half.

Treatment of intact animals for 2 days with 4 m*M* LiCl followed by 4 days with 1 m*M* LiCl (protocol A) led to the induction of multiple ectopic tentacles in the upper body region (56% of the animals, Fig. 8b). The first tentacle buds were observed 2–3 days after transfer to 1 m*M* LiCl, up to 16 ectopic tentacles developed within the next 2 days giving the animals a lampbrush morphology (Fig. 9a). Seventeen percent of these animals additionally differentiated one to three hypostomes and formed complete heads (Fig. 9b). One day after the first tentacle buds were observed, multiple feet developed in the lower body region. The ectopic heads and many of the ectopic tentacles persisted, although about 20% of the ectopic tentacles were reduced within 1 week. The variation among five independent experiments was high; in one experiment more than 80% of the animals differentiated complete ectopic heads, whereas in the next only "lampbrushes" developed. The reason for these variations could not be determined.

Multiple heads and feet can also be obtained if, during treatment with 1 m*M* LiCl, the concentration is increased for 2 days to 4 m*M* (protocol B). The ectopic head structures differentiated 2 days after reduction of the lithium concentration, but the yield of ectopic heads and

TABLE 2
REGENERATION FOLLOWING A LITHIUM PULSE TREATMENT

	Cut fragment		
	Upper	Middle	Lower
	(% formed structure in LiCl/control)		
Head	—/—	100/100	100/100
Foot	65/100	72/100	—/—
Ectopic tentacles	19/0	0/0	0/0

Note. Hydra (n = 240) were treated with 10 m*M* LiCl for 4 hr and cut into thirds. Regeneration proceeded in normal medium and was evaluated 4 days after cutting.

tentacles was only half that obtained by protocol A. Ectopic feet could already be observed on Day 4 of treatment (the last day in 4 m*M* LiCl), which corresponds to the time necessary to detect the first ectopic feet during long term treatment with 1 m*M* LiCl (Hassel and Berking, 1990).

Reduction of the treatment time in 4 m*M* LiCl from 2 to 1 day (protocol C) also showed a weaker effect. No ectopic heads formed and ectopic tentacles (up to five) developed only in regions close to the original head. Such ectopic head structures and up to three ectopic feet differentiated in about half of the animals. Transfer of the animals to normal medium had no additional effect.

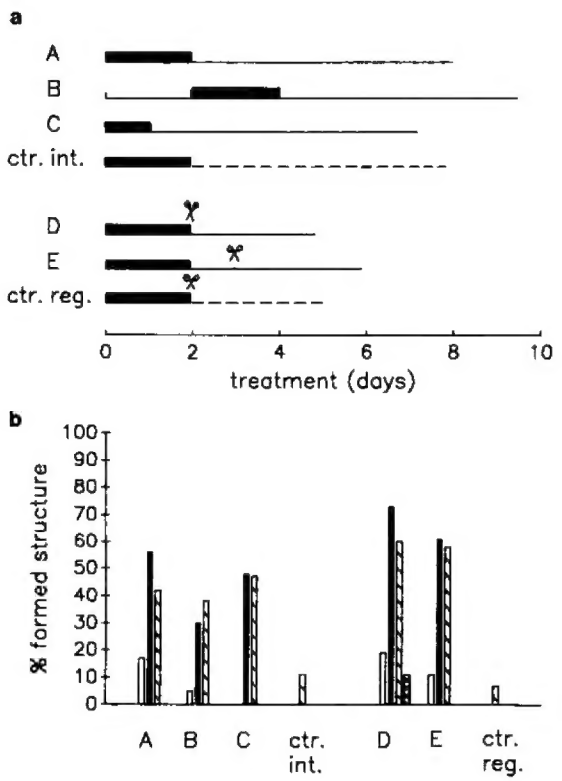

FIG. 8. Treatment protocols and results of long term treatment including variations of the lithium concentration. Five sets of experiments (A–E) were carried out. (a) Treatment protocols: In A–C intact animals were used and treated with 4 m*M* LiCl (bold line), 1 m*M* LiCl (slim line), and normal medium (dotted line) as indicated. A (n = 110), B (n = 60), C (n = 60). The control for intact animals (ctr. int., n = 60) was incubated for 2 days in 4 m*M* LiCl and then transferred to normal medium. For the regeneration experiments D and E intact animals (n = 70 each) were incubated as indicated and cut at 30 and 90% body length (scissors) either at the end of the 4 m*M* LiCl treatment (D) or after 1 day in 1 m*M* LiCl (E). Control animals (ctr. reg., n = 70) were cut in 4 m*M* LiCl and transferred immediately to normal medium. (b) Evaluation of all experiments was carried out 4 days after cutting (D, E) or the last change in the LiCl concentration (A–C). Ectopic heads in the upper body region (open bar), ectopic tentacles/lampbrush morphology (closed bar), ectopic lateral feet (hatched), altered polarity in regenerates (crosshatched).

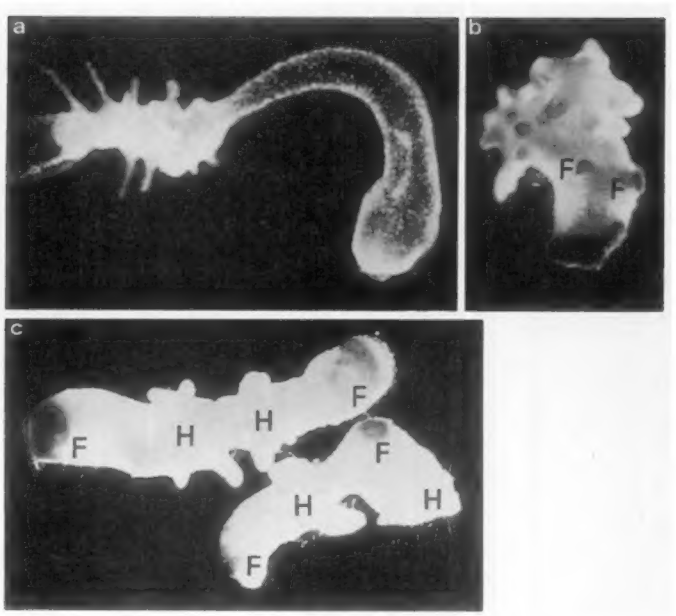

FIG. 9. Formation of ectopic heads and feet in intact and regenerating hydra after reducing the LiCl concentration from 4 to 1 m*M* LiCl. The animals were treated for 2 days in 4 m*M* LiCl and for a further 6 days in 1 m*M* LiCl. (a) Lampbrush morphology of intact animals without peroxidase staining, (b) intact animal with two ectopic heads and feet (F) after peroxidase staining. (c) Peroxidase-stained regenerating animals with severe perturbations of polarity. These animals were cut at 10 and 90% body length after 2 days in 4 m*M* LiCl and regenerated in 1 m*M* LiCl. Ectopic feet (F), ectopic head structures (H).

In a control experiment, intact animals were treated for 2 days in 4 m*M* LiCl and then transferred to normal medium. None of these animals (n = 60) developed ectopic heads or tentacles, but 7 animals differentiated one or two ectopic feet in the gastric region.

Regenerating animals gave very similar results. Animals that were cut at 30 and 90% body length according to protocols D and E regenerated supernumerary heads and tentacles at the upper cutting site. Multiple feet differentiated in the region below, but no bipolar heads or feet formed (see Fig. 9b).

In animals cut in 4 m*M* LiCl according to protocol D 8 of 70 animals showed severe perturbations of polarity (Fig. 9c). These animals differentiated either bipolar feet at the cut edges plus heads or tentacles in between or alternating structures, which persisted for at least 2 weeks.

In a control experiment hydra (n = 70) were treated and cut as in protocol D and transferred to normal medium. Seven percent of these animals developed ectopic feet, but no ectopic tentacles formed.

DISCUSSION

The time course and characteristics of the lithium effects lead us to the conclusion that lithium interferes

with pattern forming processes in *H. vulgaris* without destroying the basal differentiation potentials. The main pattern forming systems of hydra respond to lithium with a rapid inhibition of the head forming system and a slower activation of the foot formation system. Removal of the ion or reduction of its concentration induces a rapid and transient release of head formation signals, which is restricted to the upper body region.

Inhibition of Head Formation

Lithium inhibition of head formation in mid gastric and proximal thirds is a rapid effect, which is influenced by the axial position and the size of the regenerate (Fig. 5, Table 1). The fact that head formation is inhibited more completely and with shorter treatment times in proximal than in distal pieces (Figs. 1 and 5) could be due to a higher sensitivity of proximal cells to lithium or simply to a lower head formation potential of this tissue (Wolpert *et al.*, 1974; MacWilliams, 1983). Pretreatment in addition to post-treatment with lithium strikingly enhances these inhibitory effects (compare Figs. 5 and 6). Nevertheless, a weak head activation potential still persists in distal tissue in the presence of lithium since long tissue fragments (Table 1) differentiate rudimentary heads. The size of a regenerating head is normally determined by the size of the regenerate (Berking and Schindler, 1983). The fact that the rudimentary heads correspond in size to much smaller regenerates suggests that lithium-treated tissue produces only poor signals required for head regeneration.

Increase in the Foot Forming Potential

The increase in foot forming potential in lithium-treated tissue is slow compared to the inhibitory effects of lithium on head formation. It takes 3–4 days of lithium treatment before presumptive head tissue is transformed into foot tissue (Fig. 1). These 3–4 days, interestingly, correspond roughly to the time necessary to abolish the intrinsic memory of tissue polarity. Grafting a head to the foot end or vice versa stably reverses the tissue polarity after 4 days (Wilby and Webster, 1970a,b). However, in contrast to these grafting experiments, tissue polarity is not stably transformed by lithium ions. Removal of lithium leads to a sudden breakdown of the elevated foot forming potential and almost complete restoration of head formation (Fig. 6).

Ectopic feet differentiate only if lithium treatment is continued for at least 6 hr after cutting. This leads us to the conclusion that the decision to form a head or a foot is made during a lithium-sensitive phase of 5–6 hr after cutting. The time course is in good accordance with results of Webster and Wolpert (1966), Berking (1979), and MacWilliams (1983), who showed that head activation increases during the first 3–7 hr of regeneration.

Despite the high foot forming potential obtained by a lithium long term treatment, the ion does not abolish foot inhibition. High yields of bipolar foot regenerates and lateral patch feet are obtained only if the original foot is removed (Table 1). This indicates that the old foot still provides an inhibitory signal (Webster, 1971; Cohen and MacWilliams, 1975; Grimmelikhuijzen and Schaller, 1977). We cannot yet decide if ectopic feet exhibit a weaker foot inhibition or if their differentiation in close vicinity is due to their simultaneous appearance within a short time span. Only well-developed feet exhibit foot inhibition, whereas developing structures do not.

The fact that foot formation is promoted and head formation inhibited during long term treatment with lithium indicates a decrease of the positional value. Nevertheless, the head forming capacity is not destroyed. A change in the lithium concentration from 4 to 1 m*M* during long term treatment induces ectopic heads in the upper body region. This is in complete contradiction to the postulate that lithium decreases the positional value.

The ion can also increase the positional value if it is applied as a pulse. In all the experiments, where the lithium concentration was changed early during regeneration or during the incubation of intact animals (Table 2, Figs. 5, 8, and 9), ectopic head structures differentiated in the upper body region. This regional restriction of lithium-induced ectopic head formation is clearly different from the ectopic head formation induced by diacylglycerol in mid gastric tissue (Müller, 1989) and suggests that the spatial distribution of the head forming potential is not altered by lithium, but rather that there are more activating signals from already present sources.

Possible Mechanisms of the Lithium Action

The results with lithium are puzzling and cannot yet be put in a simple logical scheme. Nevertheless, there are some indications as to possible mechanisms of the lithium effects. It is unlikely that lithium itself is the morphogenetic agent as the capacity to form a foot increases slowly and lithium is supposed to enter cells quickly via Na^+ channels (Holstein-Rathlou, 1990). The lithium effects therefore are probably generated by indirect processes.

Possible targets for lithium are the two main second messenger systems in animal cells, the adenylate cyclase system, and the phosphatidylinositol (PI) cycle

(for review see Berridge *et al.*, 1989), which transmit signals that induce and control proliferation and differentiation. Both are affected by the ion: 10 m*M* LiCl interferes with noradrenaline-induced adenylate cyclase activity, whereas low lithium concentrations do not (Thams and Geissler, 1980). Low lithium concentrations are known to inhibit G-protein receptor coupling (Avissar *et al.*, 1988) and inositol mono- and bisphosphatases of the PI cycle with a K_i of 1 m*M* (Gee *et al.*, 1988; Ragan *et al.*, 1988). The inhibition of the phosphatases leads to a depletion of inositol and the subsequent inactivation of this important second messenger pathway (Hallcher and Sherman, 1980); metabolites upstream of the blocked enzymes accumulate. Recent experiments show that the PI cycle in *H. vulgaris* is strongly affected by lithium treatment (manuscript in preparation), and the PI cycle might well play a key role in the transmission or generation of signals that support the commitment to head or foot differentiation.

It is tempting to assume that head and foot activation levels operate through the PI cycle in opposite directions: DG, a possible activator of the PI cycle, induces head formation, and lithium, a possible inhibitor of the PI cycle, blocks head formation and supports foot formation. The induction of head tissue following lithium pulse treatment can also be explained, if we assume that Li^+ leads to an accumulation of PI metabolites upstream of the blocking sites. Their release after removal of the blocking agent could result in a transient overactivation of this second messenger pathway. The main problem with such a hypothesis is that the striking effects of DG observed in *H. magnipapillata* are only weak in *H. vulgaris* (Müller, unpublished results). Moreover, the foot inducing effect of lithium is restricted to *H. vulgaris*. None of the strains *H. magnipapillata wt 105* or *sf1* nor *H. viridissima* develops ectopic feet, although the induction of ectopic head structures by a lithium pulse is possible also in *H. magnipapillata* (unpublished results).

We therefore propose that there are at least two targets for the morphogenetic effects of lithium in hydra: one responds rapidly to changes in the lithium concentration and controls head formation, whereas the other requires the long term presence of the ion to induce foot formation.

Although the molecular basis of the lithium effects in hydra is still unknown, our regeneration experiments provide keys for biochemical investigations which finally should help to explain how pattern formation in hydra works.

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